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Total Phenolic Content and Antioxidant Activity of Some Medicinal Aromatic Plants and Their Constituents

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ABSTRACT

The total phenolic content and related radical scavenging activity of essential oils of five medicinal plants *Cymbopogon martini*, *Apium graveolens*, *Pimpinella anisum*, *Mentha piperita* and *Artemisia annua* were analyzed. The total phenolics were analyzed by the Folin-Ciocalteau assay while the antioxidant activity of the essential oils was analyzed by the 2,2[1]-diphenyl-1-picrylhydrazyl radical (DPPH) assay. There was a range of phenolic concentrations in the studied plant essential oils, whose values ranged from 12.69 to 55 µg (per 100 mg of gallic acid GA equivalent) as measured by the Folin-Ciocalteau assay. The essential oil of *A. graveolens* exhibited a strong (92.8%) radical scavenging effect at 50 µL. Essential oils of *C. martini* and *M. piperita* also showed a significant radical scavenging effect, which is comparatively better than BHA, a commonly used synthetic antioxidant.

Keywords: aromatic plant essential oils, pure components, radical scavenging activity, total phenols

INTRODUCTION

Antioxidants are substances which, when added to food products – particularly to lipid containing foods – can increase the shelf life of the food materials. The use of synthetic antioxidants such as Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT) and propyl gallate (PG) have been restricted in foods as these antioxidants are suspected to be carcinogenic (Mahdavi and Salukhe 1995). Therefore, the importance of the search for antioxidants from a natural plant origin has increased in recent years (Singh *et al*. 2004, 2006). Antioxidants also play an important role in human health. The imbalance between oxidants and antioxidants in our biological system has been suggested to be the cause of ageing and various diseases in humans. In modern Western medicine, the balance between oxidation and antioxidation is believed to be a critical concept maintaining a healthy biological system (Frankel *et al*. 1995; Tiwari 2001). A general recommendation to consumers is to increase the intake of food rich in antioxidant compounds (polyphenols, carotenoids) due to their well-known healthy effects. Many plants have been identified as having potential antioxidant activities and their consumption has been recommended (Friedman *et al*. 1986; Lee and Shibamoto 2000). Bioactive phenols, especially bioflavonoids are very interesting as antioxidants because of their natural origin and ability to act as efficient free radical scavengers (Hetrog *et al*. 1993, 1995; Langley-Evans *et al*. 2000). There are reports in the literature where essential oils of the seeds of *Mentha piperita* and *Apium graveolens* have shown antioxidant activity (Mimica-Dukic *et al*. 2003; Pendry *et al*. 2003). Antimicrobial and insecticidal activities of *Cymbopogon martini* have also been reported in the literature (Mohan *et al*. 2003; Prashar *et al*. 2003). Shakil *et al*. (2000, 2004) also reported the insect growth regulatory and nematicidal activity of the essential oil from *Artemisia annua*. Although the hydroxyl radical scavenging activity of *Pimpinella anisum* has been reported (Murcia *et al*. 2004) there is no previous report on such activity from the leaves of *C. martini* and *A. annua.* The antioxidant activities of water and alcohol extracts of seeds of anise (*Pimpinella anisum* L) and dill (*Anethum graveolens* L) were investigated (Mohammad Al-Ismail and Aburjai 2004). In the acidic fractions of Italo-Mitcham black peppermint oil (*Mentha* × *piperita* L.), some uncommon phenols, carboxylic acids and lactones have been identified, and reported (Näf and Velluz 1998). In addition selective extraction of oxygenates from savory and peppermint using subcritical water has been reported (Kubátová *et al*. 2001) Insofar as the antioxidant activity of the leaves of the above plants is concerned, there is no report whatsoever to the best of our knowledge. We thus undertook the measurement of total phenolics and antioxidant activity of plant essential oils of *A. annua C. martini*, *A. graveolens*, *P. anisum* and *M. piperita* and their constituents in the present study.

MATERIALS AND METHODS

Chemicals

2,2[1]-diphenyl-1-picrylhydrazyl radical (DPPH), α-terpineol, limonene, farnesol, *p*-cymene, linalool and eugenol standards were bought from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Cineole was from Acros (New Jersey, USA) and butylated hydroxy anisol (BHA) was purchased from Thomas Bakers Chemicals, Ltd., Mumbai, India. Folin-Ciocalteau reagent was brought from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and gallic acid (GA) was from Loba Chemie Pvt. Ltd., Mumbai, India. All solvents used were of analytical grade.

Isolation of the oil

The different plant materials were obtained from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi. The leaves of *A. annua* were obtained from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow. The leaves were dried at room temperature (35°C) till the leaves could be plucked by hands and ground using a domestic model electronic mixer and the powdered plant material were subjected to continuous single hydrodistillation in a Clevenger type apparatus till the oil stopped coming. It took 5 h for completion. The estimated oil yield varied from 0.09 to 0.13%. Essential oils were dried over anhydrous sodium sulphate and the samples were stored at 4°C before use.

Received: 5 July, 2006. Accepted: 10 September, 2006.

Short Communication

Measurement of total phenolics

Total phenolic concentrations were measured using the Folin-Ciocalteu assay (Asami *et al.* 2003). Briefly, 5 mL of distilled water, 1-5 mL of sample, and 1 mL of Folin-Ciocalteau reagent were mixed and allowed to stand at room temperature. Then, 10 mL of 7% sodium carbonate solution was added, followed by the addition of distilled water filled to volume. Solutions were mixed and allowed to stand at room temperature for 30 min. Total phenolic content was standardized against GA and expressed as µg per 100 mg of GA equivalents (GAE). The estimation of phenolic compounds in the essential oils was carried out in triplicate, and the results were averaged.

Antioxidant activity in terms of DPPH assay

Different amounts of various essential oils, pure components (50-250 µL) and BHA (100 ppm, 50-250 µL) were added to different test tubes. Five milliliters of 0.1 mM methanolic solution of DPPH was added to these test tubes and the contents were shaken vigorously. The tubes were allowed to stand at room temperature for 20 min in the dark (Blios 1958). The control was prepared as above without any essential oil, and methanol was used for the baseline correction. The absorbance of the samples was measured at 517 nm by a UV-VIS spectrophotometer Specord 200 Analytik Jena GmbH, Germany. Radical scavenging activity was calculated using the following formula: Percent radical scavenging activity = (Control OD - sample OD)/ Control OD × 100.

Statistical analysis

For the essential oil, three samples were prepared for assays of every antioxidant attribute. The data were presented as the mean ± standard deviation of three determinations (data were not shown). Statistical analyses were performed using a one-way analysis of variance. A probability value of $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

There was a range of phenol concentrations in the studied plant essential oils analyzed as shown in **Table 1**. The values varied form 12.69 to 55 µg (per 100 mg of GA equivalent) as measured by the Folin-Ciocalteau assay. It is well known that polyphenols are widely distributed in the plant kingdom and that they are sometimes present in surprisingly high concentrations (Harbone 1993; Catterall *et al*. 2000; Visioli *et al.* 2002; Gramza *et al*. 2006). Among the medicinal plant essential oils *Mentha piperita* had the lowest phenolic content (12.69 µg) while *Apium graveolens* had the highest (55 µg) (**Table 1**). According to Singleton *et al*. (1965) various phenolic compounds have different responses in this assay. The structural features of the phenolic compounds (Frankel *et al*. 1995) may also be responsible for the antioxidant activity of particular essential oils.

Table 1 Phenolic content of different essential oils as per gallic acid standard.

Plant material	Total phenolics (µg/100 mg)[a]
Artemisia annua	27.33
Cymbopogon martini	29.32
Pimpinella anisum	22.00
Mentha piperita	12.69
Apium graveolens	55.00

[a] microgram gallic acid equivalent per 100 g.

Fig. 1 shows the DPPH radical scavenging activity of various plant essential oils along with synthetic antioxidant BHA, the positive control in this reaction. Among the studied essential oils, *A. graveolens* exhibited strong (92.8%) radical scavenging effect at 10 µL/mL, which is much better than that reported by Pendry *et al*. (2003). The essential oils of *Cymbopogon martini* and *M. piperita* also showed significant radical scavenging effect, comparatively better than BHA. The essential oil of *A. graveolens* has a higher content of total phenols and there is a significant relation between the total phenols and radical scavenging activity. But for other essential oils there is no strong relation between radical scavenging activities and phenolic content. This proved that the antioxidant activity does not solely depend upon the phenolic content present in the samples but that other components may also have a significant contribution.

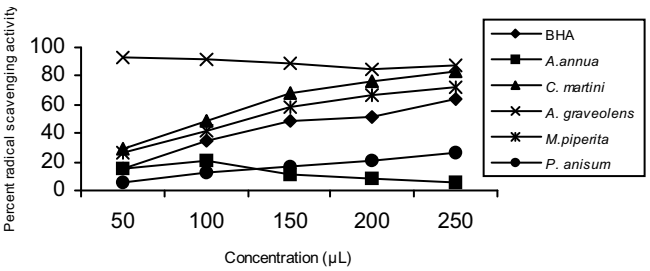

Fig. 1 Radical scavenging activity of various plant essential oils.

In order to confirm the above assumption, we also measured the radical scavenging activities of pure components commonly present in plant essential oils (**Fig. 2**). The phenolic compound eugenol showed the highest radical scavenging activity, followed by other compounds such as limonene and farnesol (**Fig. 2**). In addition, cineole, α-terpineol, *p*-cymene and linalool also exhibited radical scavenging activity. This showed that the radical scavenging effect of tested essential oils is dependent on the synergistic effects of different components present in them.

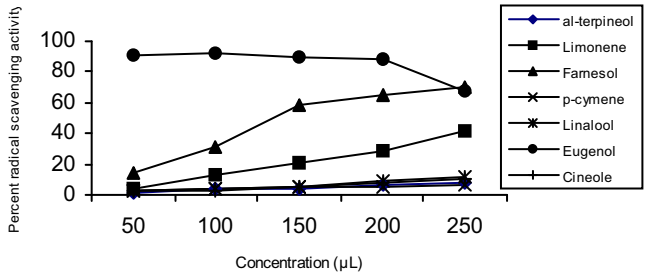

Fig. 2 Radical scavenging activity of pure components.

Generally, an increase in concentration of essential oil led to the increase of radical scavenging activity for the *M. piperita*, *P. anisum* and *C. martini* essential oils. But this was not true for the other essential oils studied in the present study. For *A. graveolens* and *A. annua* essential oils, above a certain concentration (150 µL onwards), radical scavenging activity decreased with an increase in dose concentration. Our report indicates that at a certain concentration (150 µL onwards), the availability of the hydrogen atom to scavenge the DPPH radical might be hindered. It has already been reported that the antioxidant activity of phenolic compounds depends upon their molecular structures, that is, on the availability of phenolic hydrogens and on the possibility of stabilization of the resulting phenoxyl radicals formed by a hydrogen donation (Catherine *et al*. 1996; Ramarathnam *et al*. 1997).

CONCLUSION

The results of the present work indicate that the studied essential oils possess moderate to strong free radical scavenging activity. However, further investigation of individual phenolic and other components, *in vivo* and antioxidant activity mechanism is warranted. These studies can be useful as a starting point for further applications of these essential oils and their constituents in food and pharmaceutical preparations.

ACKNOWLEDGEMENTS

The authors thank the Head, Division of Agricultural Chemicals, IARI, New Delhi for allowing us to carry out the research work. We thank Dr. Rajesh Kumar, Division of Agricultural Chemicals for useful discussion. Technical assistance of Mr. Jitendra Mohan, NBPGR, New Delhi is also acknowledged.

REFERENCES

Asami DK, Hong YJ, Barrett DM, Alyson EM (2003) Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry and corn grown using conventional, organic and sustainable agriculture practices. *Journal of Agricultural and Food Chemistry* **51**, 1237-1241

Blios MS (1958) Antioxidant determination by the use of stable free radicals. *Nature* **181**, 1199-1200

Catherine A, Rice Evans CA, Nicholas JM, George P (1965) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine* **20**, 933-956

Catterall F, Souquet JM, Cheynier V, de Pascual-Teresa S, Santos-Buelga C, Clifford MN, Ioannides C (2000) Differential modulation of the genotoxicity of food carcinogens by naturally occurring monomeric and dimeric polyphenolics *Environmental and Molecular Mutagenesis* **35**, 86-98

Frankel EN, Waterhouse AL, Teissedre PL (1995) Principal phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of low-density lipoproteins. *Journal of Agricultural and Food Chemistry* **43**, 890-894

Friedman LA, Kimbail AW (1986) Coronary heart disease mortality and alcohol consumption in Framingham. *American Journal of Epidemiology* **24**, 481-489

Gramza A, Khokhar S, Yoko S, Gliszczynska-Swiglo A, Hes M, Korczak J (2006) Antioxidant activity of tea extracts in lipids and correlation with polyphenol content *European Journal of Lipid Science and Technology* **108**, 351-362

Harborne JB (1993) *New Naturally Occuring Plant Polyphenols, Polyphenolic Phenomena*, Paris, Scalbert, INRA

Hetrog MGL, Hollman PCH, Van de Putte B (1993) Content of potentially anticancerogenic flavonoids of tea infusions, wines and fruit juices. *Journal of Agricultural and Food Chemistry* **41**, 1242-1246

Hetrog MGL, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F (1995) Flavonoid intake and long-term risk of coronary disease and cancer in the seven countries study. *Archives of International Medicine* **155**, 381-386

Kubátová A, Lagadec AJM, Miller DJ, Hawthorne SB (2001) Selective extraction of oxygenates from savory and peppermint using subcritical water. *Flavour and Fragrance Journal* **16**, 64-73

Langley-Evans SC (2000) Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay. *International Journal of Food Science and Nutrition* **51**, 181-188

Lee KG, Shibamoto T (2000) Antioxidant properties of aroma compounds isolated from soybean and mung beans. *Journal of Agricultural and Food Chemistry* **48**, 4290-4293

Mahdavi DL, Salukhe DK (1995) Toxicological aspects of food antioxidants. In: Mahdavi DL, Deshpande SS, Salukhe DK (Eds) *Food Antioxidants*, Marcel Dekker, New York, 267 pp

Mimica-Dukic, Bozin B, Sokovic M, Mihajlovic B, Matavulj M (2003) Antimicrobial and antioxidant activities of three *Mentha* species essential oils. *Planta Medica* **69**, 413-419

Mohan K, Ebenezar EG (2003) Management of leaf blight disease of onion by palmarosa oil. *Indian Perfumer* **47**, 169-171

Mohammad Al-Ismail K, Aburjai (2004) Antioxidant activity of water and alcohol extracts of chamomile flowers, anise seeds and dill seeds. *Journal of the Science of Food and Agriculture* **84**, 173-178

Murcia MA, Egea I, Romojaro F, Parras P, Jimenez AM, Martinez-Tome M (2004) Antioxidant evaluation in dessert spices compared with common food additives. Influence of irradiation procedure. *Journal of Agricultural and Food Chemistry* **52**, 1872-1881

Näf R, Velluz A (1998) Phenols and lactones in Italo-mitcham Pepper mint oil. *Flavour and Fragrance Journal* **13**, 203-208

Pendry B, Busia K, Bell CM (2003) Evaluation of the antioxidant activity of selected non-food plants traditionally used in the treatment of arthritis. *Focus on Alternative and Complementary Therapy* **8**, 528-529

Prashar A, Hili P, Veness RG, Evans CS (2003) Antimicrobial action of palmarosa oil (*Cymbopogon martinii*) on *Saccharomyces cerevisiae*. *Phytochemistry* **63**, 569-575

Ramarathnam N, Ochi H, Takeuchi M (1997) Antioxidant activity defense system in vegetable extarcts. In: Shahidi F (Ed) *Natural Antioxidants. Chemistry, Health Effects and Applications*, AOCS Press, Champaign, IL, USA, pp 76-87

Shakil NA, Prasad D, Saxena DB, Gupta AK (2004) Nematicidal activity of essential oils of *Artemisia annua* against root knot and reniform nematodes. *Annals of Plant Protection Sciences* **12**, 403-408

Shakil NA, Saxena DB, Singh S, Gupta AK, Subrahmanyam B (2000) Insect growth regulatory activity of *Artemisia annua*. *Pesticide Research Journal* **12**, 36-40

Singh G, Marimuthu P, Catalan AN, De Lampasoma MP (2004) Chemical, antioxidant and antifungal activities of volatile oil of black pepper and its acetone extract. *Journal of the Science of Food and Agriculture* **84**, 1878-1884

Singh G, Marimuthu P, de Heluani CS, Catalan AN (2006) Antioxidant and biocidal activities of *Carum nigrum* (seed) essential oil, oleoresin and their selected components. *Journal of Agricultural and Food Chemistry* **54**, 174-181

Singleton VL, Rassi JA (1965) Calorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Ecology and Viticulture* **16**, 144-158

Tiwari AK (2001) Imbalance in antioxidant defense and human diseases; multiple approach of natural antioxidant therapy. *Current Science* **8**, 1179-1187

Visioli F, Poli A, Gall C (2002) Antioxidant and other biological activities of phenols from olives and olive oil. *Medicinal Research Reviews* **22**, 65-75